TIANexact Genotyping qPCR PreMix (Probe)

For detection of SNPs by PCR (Probe method)

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Cat. No. 4992874/4992875

Kit Contents

Contents	4992874 20 µl × 125 rxn	4992875 20 µl × 500 rxn
2 × TIANexact Genotyping PreMix (Probe)	1.25 ml	4 × 1.25 ml
50 × ROX Reference Dye	250 µl	1 ml
RNase-Free ddH_2O	2 × 1 ml	5 × 1 ml
Handbook	1	1

Storage

The TIANexact Genotyping qPCR PreMix (Probe) can be stored at -30~-15°C for one year. It should be stored immediately after receipt at -30~-15°C, protected from light. Thaw the 2 × TIANexact Genotyping PreMix (Probe) and 50 × ROX Reference Dye and mix thoroughly before use. If the 2 × TIANexact Genotyping qPCR PreMix and 50 × ROX Reference Dye are thawed but not used, it is important to thoroughly mix prior to re-freezing. The layering of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance. For frequent use, PreMix can be stored at 2-8°C for 3 months. Repeated freeze-thaw cycles should be avoided.

Introduction

TIANexact Genotyping qPCR PreMix (Probe) is a 2 × hot start PCR premix which could be used in SNP analysis (Probe method). This premix contains specific antibody modified *Taq* DNA polymerase, by which low concentration DNA template could be easily detected. The unique buffer system could efficiently eliminate the inhibition to PCR and the effect to fluorescence quenching from a lot kind of PCR inhibitors. This Kit provides an efficient and high specific method of SNP identification.

Product Features

1. "Bullet-proof" genotyping---Eliminate the influence of PCR inhibitors to SNP genotyping.
2. Fast reaction---Quick PCR reaction setup in most experiments.
3. High fluorescent signal---Low amount of primers and probes are required to perform SNP genotyping.
4. Avoid operational error---Blue indicator dye is added to monitor the experiment setup process.

Principle of Operation

1. This product can be used on qPCR and SNP analysis which uses hydrolysis probe technique. This product eliminates the inhibition effect of PCR inhibitors, especially for crude extract which have high stress resistance. This product suits for the analysis of crude extracts of plants, animals or environmental samples.
2. 2 × TIANexact Genotyping PreMix (Probe) contains specific antibody modified hot start *Taq* DNA polymerase, by which all the enzyme activity can be activated within 2-5 min at 95°C. It reduces the reaction time and maximally reduces the non-specific amplification during the PCR reaction. In addition with the optimized buffer system, this product has high specificity and amplification efficiency on the identification of sequence variation.
3. 2 × TIANexact Genotyping PreMix (Probe) includes dNTPs, enhancer and stabilizer which increase the specificity of product and reaction sensitivity. A blue indicator dye is also included in this product which simplifies the experiment setup and has no negative effect on PCR reaction and fluorescent probe. This dye could help to avoid operational error on experiment setup of large amount of small volume samples (10 μl).

Important Notes

1. Performance of reagents may reduce without mix completely. Please gently mix the reagents by inverting the tubes and centrifuge briefly prior to use. Do NOT vortex and avoid producing bubble.
2. Fluorescent probe is not included in this kit.
3. Typically, best amplification results can be obtained using a primer concentration of 300 nM and a probe concentration of 200 nM. However, for individual determination of optimal primer concentration, a primer titration from 100-900 nM and a probe titration from 100-250 nM can be performed.
4. In a 20 μl reaction volume, the amount of genomic DNA template is usually less than 100 ng.

Protocol

<1> Set up the Real-Time PCR reaction system

1. Thaw TIANexact Genotyping PreMix (if stored at -30~-15°C), 50 × ROX Reference Dye, template, primers, probes and RNase-Free ddH_2O. Completely mix and equilibrate reagents to room temperature before use.
2. All the Real-Time PCR reaction steps should be operated on ice.

Reaction system:

Component	20 μl volume	Final concentration
2× TIANexact Genotyping PreMix (Probe)	10 μl	1 ×
Forward Primer (10 μM) △1	0.6 μl	100-900 nM
Reverse Primer (10 μM) △1	0.6 μl	100-900 nM
Probe (10 μM) △2	0.4 μl	100-250 nM
Template	2 μl	-
50 × ROX Reference Dye △3	-	-
RNase-Free ddH_2O	to 20 μl	-

△1 A final primer concentration of 300 nM is optimal for most applications. Higher concentration can be used when the amplification efficiency is not favorable. If non-specific amplification is observed, the primer concentration should be reduced. For further optimization, a primer titration from 100-900 nM can be performed.

△ 2 The concentration of probe is related to the type of Real-Time PCR instruments, type of probes and type of fluorescent markers. Please check the handbook of instrument or probe for specific concentration. A final probe concentration of 200 nM is optimal for most applications. For further optimization, a probe titration from 100-250 nM can be performed.

△ 3 The optimal concentration of ROX Reference Dye for commonly used Real-Time PCR instruments:

Instrument	Final Concentration
ABI PRISM 7000/7300/7700/7900HT/Step One	5 × (e.g. 5 µl ROX/ 50 µl volume)
ABI 7500, 7500 Fast; Stratagene Mx3000P, Mx3005P and Mx4000	1 × (e.g. 1 µl ROX/ 50 µl volume)
Roche, Bio-Rad and Eppendorf instruments	No need

3. Close the tubes and mix samples gently. Brief centrifugation can be performed to collect residual liquid from the walls of the tubes.
4. Place the PCR tubes in the thermal cycler and then start the PCR cycle.

<2> Real-Time Amplification

Typically, best results are obtained using a two-step PCR. However, if two-step PCR does not yield favorable results (e.g. non-specific amplification caused by low template concentration or reduced amplification efficiency induced by low Tm value) the three-step PCR is recommended.

Two-step PCR:

Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1×	95°C	2 min △ 1	Initial denaturation	N
PCR	40×	95°C	15 sec	Denaturation	N
		60°C	30 sec △ 2	Annealing/ Extension	Y

Three-step PCR:

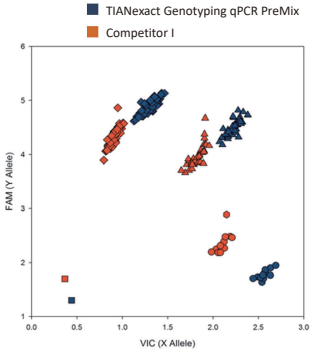
Stage	Cycle	Temperature	Time	Step	Signal Collection
Initial denaturation	1×	95°C	5 min △1	Initial denaturation	N
PCR	40×	95°C	15 sec	Denaturation	N
		50-60°C	15 sec	Annealing	N
		72°C	30 sec △2	Extension	Y

△1 Melting time depends on the length and GC content of template.

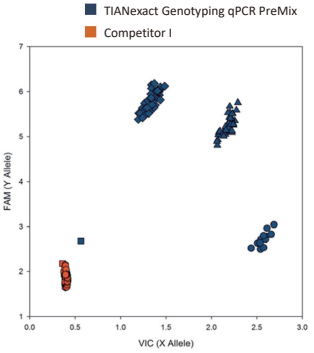
△2 Please set the extension time according to the handbook of thermal cycler.

Set to 15 sec for Roche, ABI 7500 Fast, Bio-Rad and Agilent PCR instruments.
Set to 20 sec for ABI 7900HT/7900HT Fast/ViiA 7/ StepOne/StepOnePlus.
Set to 31 sec for ABI 7000/7300.
Set to 32 sec for ABI 7500.

Reaction Example



A. In contrast with Competitor I, TIANexact shows stronger fluorescent signal and better cluster effect.



B. After the treatment with PCR inhibitor humic acid (50 ng/μl), PCR amplification of Competitor I is inhibited, but PCR amplification of TIANexact still shows accurate result.

Troubleshooting

1. Irregular melting curve with low concentration template, low fluorescent signal.

Reason	Solution
Low copy number of target DNA	Low copy number of target DNA in the reaction mix (<100) may lead to variation of copy number and irregular melting curve. Please increase the template concentration.
Compete with primer dimer	Amplification of primer dimer weakens the amplification of target DNA fragment. Please optimize the reaction condition or redesign primers to avoid the formation of primer dimer.
DNA is adsorbed by centrifuge tube	If DNA concentration is too low or the DNA is stored for long term stored after dilution, DNA may be adsorbed by tubes. Please start the PCR reaction right after dilution.

2.Low repeatability.

Reason	Solution
Instrument problem	Low repeatability caused by instrument error. Please check instrument handbook for solution.
Low sample purity	Low purity sample will lead to low repeatability.
Long term storage after dilution	If low concentration DNA solution is stored for long term, DNA may be adsorbed by centrifuge tubes. Please start the reaction right after the dilution. In addition, gradient dilution needs to be diluted from original sample liquid.
Low primer or probe quality	Avoid the difference between batches of primers, original primers of good quality can be used as positive control.
PCR condition, primer concentration or sequence is not appropriate.	Low efficient PCR may lead to low repeatability. It can be optimized by adjusting the concentration of primer and probe or the PCR condition. Reducing the annealing temperature or increasing primer concentration or increasing extension time can be applied to optimize the amplification.
Operation error	Low reaction volume may reduce the accuracy of detection. Please adjust the reaction volume according to the handbook of PCR instrument.

3. NTC amplification shows positive result.

Reason	Solution
Cross contamination	Please change reagents and ddH_2O. If there is still cross contamination, please change to new experiment environment.
Instrument setup error(multi-PCR)	Please setup the fluorescent analysis correctly when multiple probes are used, in order to avoid the signal difference caused by the cross of spectrums of different fluorescent dye.

4. Low fluorescent signal of amplification curve or the amplification curve shows zigzag.

Reason	Solution
Spectrum setup error	Given that there are differences between Real-Time PCR instruments on principle and wavelength range of light source, so luminophore and quencher need to be selected according to the handbook of PCR instruments, and the parameters of instrument need to be adjusted.
Low probe purity	Please use probes that at least purified with HPLC level, or else the residual fluorescent dye may lead to baseline drift, and then reduce the fluorescent value of amplification product.
Low probe quality	If the probe is degraded during storage, the baseline may also drift then lead to the reduction of fluorescent value. In addition, some fluorescent dye cannot be stored in buffer with EDTA. Please check the storage condition with probe supplier.
Short fluorescence collection time	For some PCR instrument, extension time need to be longer to collect enough fluorescence. If the amplification curve shows zigzag, please set the extension time to 45-60 sec.